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Short Communication

Entomopathogenic fungi recorded from the harlequin ladybird, *Harmonia axyridis*

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ABSTRACT

Entomopathogenic fungi were recorded from field samples of the harlequin ladybird *Harmonia axyridis*, an invasive coccinellid that has recently arrived in Denmark. Larvae, pupae and adults were found to be infected by *Isaria farinosa*, *Beauveria bassiana* and species of *Lecanicillium*. This is the first record of entomopathogenic fungi infecting larvae and pupae. Winter mortality due to fungal infection reached 17.9% in adults collected at one location. The larval stage was most susceptible to fungal infection, as confirmed through bioassay with *I. farinosa*.

Adults of the harlequin ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae), an invasive species originating in Asia, have been reported to be only marginally susceptible to infection by the entomopathogenic fungus *Beauveria bassiana* in laboratory infection experiments (Cottrell and Shapiro-Ilan, 2003; Roy et al., 2008). In accordance, in overwintering adults of *H. axyridis* and an indigeneous ladybird species, *Olla v-nigrum*, a substantial proportion of the latter died from infection with *B. bassiana*, whereas *H. axyridis* never succumbed to infection (Cottrell and Shapiro-Ilan, 2003). This lack of susceptibility to infection by entomopathogenic fungi is supported by the almost absent records of natural infections of this widely studied invasive ladybird species. To our knowledge, the only report is Kuznetsov (1997) who merely stated that *H. axyridis* is infected by *Beauveria* spp. in its native range in eastern Russia and that the impact of the fungus is minimal.

The harlequin ladybird has invaded much of Europe during the last decade (Brown et al., 2008), and by October 2007 it was abundant in different areas of Copenhagen (Steenberg and Harding, 2008).

From early November 2007 to mid December 2008 larvae (three samples), pupae (four samples) and adult *H. axyridis* (six samples) were collected from locations in Copenhagen. Many larvae were moribund or dead and many pupae failed to emerge as adults, particularly when collected late in the sampling period. Newly dead specimens were incubated in Petri dishes lined with moist filter paper to allow sporulation of entomopathogenic fungi. Adults were stored in a temperature cabinet (6 °C, 65% r.h., no light) until March, when mortality was recorded and dead individuals placed under moist conditions. In late May 2008, pupae of the first generation of the year were sampled from five locations; those failing to emerge were checked for fungus infection. Fungi were identified based on the morphology of conidiophores and conidia using light microscopy (Humber, 1997).

The pathogenicity of one isolate of *Isaria farinosa* collected from an infected *H. axyridis* larva was tested in a bioassay, where field-collected *H. axyridis* larvae ($n = 20$), adults of *H. axyridis* ($n = 20$) and adults of the two-spotted ladybird *Adalia bipunctata* (Coleoptera: Coccinellidae) ($n = 20$) were immersed individually for 5 s in 10 ml of a spore suspension (1.8×10^7 conidia per ml, >90% germination after 24 h at 20 °C). Control groups in similar numbers were immersed in sterile 0.02% Tween-80. Insects were kept individually in capped cups for 16 days (25 °C, 18:6 L:D, relative humidity ~100% r.h. (48 h) followed by 80% r.h. (14 d)) and fed eggs of *Ephestia kuehniella ad lib* every 2–3 days. Dead specimens were recorded at 2–3 day intervals and incubated under moist conditions to confirm infection by *I. farinosa*.

Entomopathogenic fungi were isolated from larvae, pupae and adults of *H. axyridis* and from 15 of 18 samples. Four species of mitosporic fungi were isolated: *I. farinosa*, *Beauveria bassiana*, *Lecanicillium muscarium*, *Lecanicillium lecanii* and also at least one unidentified *Lecanicillium* sp. The records of *I. farinosa* and

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Table 1
Entomopathogenic fungi recorded from different life stages of the harlequin ladybird (*H. axyridis*).

Life stage	Time period	Collected live	Dead during incubation		With fungus			Number[a] of specimens infected with			
		N	*N*	In % of collected	*N*	In % of collected	In % of dead	*Isaria farinosa*	*Beauveria bassiana*	*Lecanicillium* sp.	Mixed infection
Larvae	November–December 2007	–	89	–	22	–	24.7	16(18)	0(2)	4	2
Pupae	November–December 2007	–	221	–	30	–	13.7	17(24)	3(8)	3(6)	7
Adults	November 2007–January 2008	664	230	34.6	30	4.5	13.0	10(12)	2(4)	16	2
Pupae	May 2008	2247	196	8.7	27	1.2	13.8	17(19)	4(8)	1(5)	5
Total					109			60(73)	9(22)	24(31)	16

[a] Numbers in brackets include records from mixed infections – denotes that no data are available since most specimens were moribund or dead at the time of collection.

Lecanicillium spp. are new for adult *H. axyridis*, and larvae and pupae have not previously been found to be naturally infected by entomopathogenic fungi. Most of the *Lecanicillium* isolates could not be identified with certainty to species level as the morphology of conidiophores and conidia does not fit existing species descriptions (Zare and Gams, 2001; R.A. Humber, pers. comm.). In Table 1 all isolates belonging to this genus are pooled. *I. farinosa* was the dominant species in all life stages studied and infected 67% of the fungus-killed specimens. *Lecanicillium* sp. was markedly more common in adult *H. axyridis* than in larvae and pupae, in contrast to *I. farinosa*. Approximately 15% of specimens were infected by more than one fungus species, most frequently *I. farinosa* and *B. bassiana*. The species isolated from *H. axyridis* were also isolated from the indigenous coccinellids *Adalia bipunctata* and *Calvia quatuordecimguttata* sampled at the same time (unpublished data). While most reports on fungi in overwintering coccinellids describe only records of *B. bassiana*, Ceryngier (2000) documented the same fungal species in overwintering *Coccinella septempunctata*, and *I. farinosa* has previously been found infecting this coccinellid in what appears to be an epizootic (Pacioni and Frizzi, 1977). *Isaria* and *Lecanicillium* may have been misidentified in previous studies as they cause a white mycosis like *B. bassiana*.

In average, 4.5% of the overwintering *H. axyridis* died from fungus infection (range: 1.9–17.9%). The literature on naturally occurring entomopathogenic fungi in coccinellids is scarce and mostly refers to overwintering adults. Based upon our results, fungal prevalence in overwintering *H. axyridis* adults varies, but is not necessarily lower than infection levels found in adults of other coccinellid species (e.g. Iperti, 1964; Lipa et al., 1975). In comparison, a relatively low fungal prevalence was found in pupae collected in May (1.2% infected overall, range 0.5–3.8%).

We only have little data on the prevalence in live collected larvae and pupae sampled in November–December. In one sample of 25 last-instar larvae 17 died and of these seven were infected with fungi, while no adults emerged from one sample of 15 pupae, four of which supported sporulating fungus. Among larvae found dead or dying soon after collection, between 11% and 62% were infected by fungus. Similarly, 7–23% of dead pupae collected in late autumn were infected. The mycosis in pupae often appeared to originate from the shed larval skin that remains where pupae attach to the substrate, indicating that infection took place in the larval stage. In two pupae the fungus was visible on the ventral side of the insect already at the time of collection. Naturally occurring fungi in the larval and pupal stages of coccinellids have hardly been described at all, although Yinon (1969) described larvae of *Chilochorus bipustulatus* succumbing to an unidentified fungus on tree trunks at the onset of winter.

In the infection experiment all larvae in the control pupated and later emerged as adults, while all larvae treated with *I. farinosa* died from fungus infection. For adult ladybirds, no mortality was seen in the control treatment, and the two specimens (one *H. axyridis*, one *A. bipunctata*) that died after fungus treatment did not support sporulating fungus. This preliminary data shows that larvae are more susceptible to infection than adults, and supports the finding of a high proportion of fungus-infected larvae in the field. The high infection levels in larvae and pupae collected very late in season could however also reflect that these individuals may be stressed by low temperatures and scarcity of food. It remains to be seen whether the fungi documented here play a role in the natural regulation of *H. axyridis*, not only in the adult overwintering stage but notably in the immature stages in spring, summer and autumn.

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